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In vivo multimodal imaging of stem cell transplantation in a rodent model of Parkinson's disease

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ABSTRACT

Stem cell therapy in the nervous system aims to replace the lost neurons and provide functional recovery. However, it is imperative that we understand the *in vivo* behaviour of these cells post-implantation. We report visualisation of iron oxide labelled bone marrow-derived stem cells (BMSCs) implanted into the *striatum* of hemi-parkinsonian rats by magnetic resonance imaging (MRI). Functional efficacy of the donor cells was monitored *in vivo* using the positron emission tomography (PET) radioligand [^{11}C]raclopride. The cells were visible for 28 days by *in vivo* MRI. BMSCs provided functional recovery demonstrated by a decreased binding of [^{11}C]raclopride. Although, histology confirmed the persistence of donor cells, no tyrosine hydroxylase positive cells were present. This suggests that BMSCs may have a limited paracrine effect and influence functional recovery. We demonstrate, using multimodal imaging, that we can not only track BMSCs but also establish their effects in a pre-clinical model of Parkinson's disease.

1. Introduction

Neurodegenerative diseases such as Parkinson's disease (PD) are characterised by cell loss and are thus ideal candidates for stem cell replacement therapy. Although substantial improvements result from the systemic administration of L-DOPA or dopamine agonists, such pharmacological interventions do not address the aetiology of the disease, provide a permanent remedy or prevent progression of the degenerative process (Snyder and Olanow, 2005). Implantation of stem cells will provide a more constitutive and relevant solution. This realisation has prompted a renewed interest in stem cells, which may serve as a replenishable source of cells for the treatment of neurodegenerative disorders. The neurotoxin, 6-OHDA was used to induce dopaminergic degeneration in the *striatum*. Whilst other models of PD are available (e.g. MPTP, knock-in mice), this method is more progressive and, in this case, more closely mimics the human form of PD (Schwarting and Huston, 1996b).

A clinically relevant strategy may be to implant bone marrow-derived mesenchymal stem cells (BMSCs) that are constitutively capable of neural differentiation and cytokine secretion. Allowing the cells to develop within the PD-affected brains, yields cells whose phenotypes, numbers, locations, and regulation are determined by the interplay of donor elements and the local host *milieu*. A consequence of such donor–host interaction would result in a more pertinent homeostasis. We hypothesized that the BMSCs-based approach might better mitigate some of the limitations of previous strategies, where pre-programmed partially differentiated cells did not provide functional recovery (Brederlau et al., 2006). Furthermore, BMSCs can be used as autologous or allogeneic therapies, reducing the need for immunosuppression. They are also relatively easy to harvest in the clinic, with procedures used routinely in bone marrow donations.

Due to the seamless integration into the host parenchyma, and migration over long distances, cell grafts cannot be detected based on their mass morphology. To monitor cell migration and positional fate after transplantation, current methods use either reporter genes or chimeric animals. These methods are cumbersome, involve sacrifice of the animal and removal of tissue for histological procedures, and cannot be translated to human studies. Furthermore, this approach lacks the temporal analysis of the donor cells, so in practice its uses are limited. Thus, in order to assess the efficacy of stem cell therapies in experimental and clinical studies, there is a need for the development of non-invasive imaging methodolo-

Abbreviations: 6-OHDA, 6-hydroxydopamine; DMEM, Dulbecco's modified Eagle medium; GFAP, glial fibrillary acidic protein; IB4, Isolectin B4; IODEX-TAT-FITC, dextran coated iron oxide-TAT-fluorescein isothiocyanate; L-DOPA, levadopa; MRI, magnetic resonance imaging; BMSCs, mesenchymal stem cells; PBS, phosphate-buffered solution; PD, Parkinson's disease; PET, positron emission tomography; TH, tyrosine hydroxylase; USPIO, ultrasmall superparamagnetic iron oxide; VOI, volume-of-interest.

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gies to monitor the bio-distribution of implanted cells and measure their functional recovery (Kirik et al., 1998). Thus, imaging can play a central role in shaping the future of cell-based therapy of PD.

However, no single imaging modality can provide all the information required to track transplanted stem cells and monitor their functional effects; hence, there is a necessity for combining complementary imaging methodologies. Indeed, the combination of magnetic resonance imaging (MRI) and positron emission tomography (PET) allows the acquisition of anatomical, physiological and metabolic information, all from the same subject.

The monitoring of cellular grafts, non-invasively, is an important aspect of the ongoing efficiency and safety assessment of cell-based therapies. For transplanted stem cells to be visualised and tracked by imaging technologies, they need to be tagged so that they are 'visible'. The use of intracellular contrast agents ensures that the cells are easily distinguishable from the host tissue whilst preserving long-term cellular viability and function. This study used bifunctional contrast agent dextran coated iron-oxide nanoparticles (IODEX-TAT-FITC) to pre-label the stem cells prior to MR imaging. One of the advantages of using the bifunctional contrast agent is the ability to confirm the cell labelling *in vitro* using fluorescence microscopy.

However, contrast agents are required to cross cellular membranes and deliver sufficient amounts for imaging, whilst achieving intracellular retention *in vivo*. There are a number of transfection agents available; however this study used the HIV-derived transactivator of transcription (TAT) peptide as a transfection agent, conjugated to the dextran coating of the IO particles. TAT-peptide, a domain of the HIV TAT protein, has been shown to enhance cell uptake of various attached biomolecules (Zhang et al., 2003). The use of a TAT-peptide system to transfect cells *in vitro* not only results in reproducible cellular labelling with a higher labelling efficiency, but also requires a shorter incubation period (Josephson et al., 1999).

In addition, radionuclide-imaging modalities such as PET can monitor neurochemical events in the *striatum* (Pellegrino et al., 2007). As PD involves the loss of a specific cell population, namely dopaminergic neurons, it is particularly well suited for assessment by PET imaging. The PET radioligand [^{11}C]raclopride, a D_2 receptor antagonist, competes for receptor occupancy and indirectly reports on the release of endogenous dopamine (Hume et al., 1992).

Furthermore, it has been shown that changes in the neurochemistry of the basal ganglia may be detected by PET imaging long before clinical symptoms develop (Ishida et al., 2005). Changes in [^{11}C]raclopride and [^{18}F]fluorodopa binding were evident within three days after 6-OHDA lesioning, whereas behavioural changes assessed by methamphetamine-induced rotations developed much later. This study also demonstrated an increased binding of [^{11}C]raclopride in the 6-OHDA lesioned area. This clearly shows that PET imaging is more sensitive to dopamine imbalance than behavioural testing and may therefore be more suitable in assessing functional recovery following cell implantation in PD models.

Thus, the combination of MRI and PET provides both anatomical spatial resolution and biochemical sensitivity, respectively for the assessment of dopaminergic function. Here, we demonstrate for the first time, in a pre-clinical model of PD transplanted with stem cells, that combining MRI with PET can provide longitudinal information on the delivery of stem cells as well as monitoring recovery of dopaminergic function.

2. Materials and methods

All animal work was carried out in accordance with Home Office regulations under the UK Animals (Scientific) Procedures Act 1986.

A timeline of various experimental protocols is outlined in Fig. 1.

2.1. Isolation and culture of rat BMSCs

Bone marrow-derived BMSCs were harvested from femurs and tibias of adult male Sprague–Dawley rats (150–200 g, Harlan, UK). The cells were seeded in high-glucose Dulbecco's modified Eagle's medium with L-glutamine supplemented with 10% foetal bovine serum and 25 μg/mL gentamicin on previously poly-L-lysine coated flasks. Culture media was replaced after two days and changed twice a week thereafter. Haematopoietic and other non-adherent cells were removed during medium changes. After 10 to 14 days of primary cultivation, the adherent cells were nearly 80% confluent. The cells were dissociated with 0.25% trypsin and 1 mM EDTA and re-plated to expand the cells through successive passages. The mesenchymal lineage of the rat BMSCs was confirmed by their ability to readily differentiate into adipocytes and osteoblasts (Zhao et al., 2005) (data not shown).

2.2. Animal model

Healthy adult (250–300 g) male Sprague–Dawley rats (Harlan, UK) were acclimatized for a week on a regular diet and equal light/dark cycle. Rats were given *ad libitum* access to food and water. 25 mg/kg Desipramine was administered 30 min prior to surgery to prevent the degeneration of noradrenergic neurons in the *striatum*. Anaesthesia was maintained using isofluorane (2%) and oxygen (2 L/min). Sterereotactic surgery took place on a Kopf small animal stereotactic frame. Thermoregulation was monitored with a rectal probe and kept constant at 37 °C with a feedback-controlled warm blanket (Harvard Apparatus, Edenbridge, UK). 6-Hydroxydopamine (6-OHDA) was injected into the right *striatum* at a dose of 16 μg, in a volume of 4 μL at a rate of 1 μL/min, using a micropipettor (Harvard Apparatus, Edenbridge, UK). The stereotactic co-ordinates for injection were based on Rat Brain Atlas (Paxinos and Watson, 2006) with reference to the *bregma*, using the following co-ordinates; anterior–posterior = 0.0 mm, lateral–medial = −2.0 mm, dorsal–ventral = −6.3 mm.

2.3. Behavioural scoring

The PD lesion was assessed by behavioural testing, one week post-lesioning (Fig. 1), using a validated quantitative time-sampling method based on methamphetamine-induced rotations (Schwarting and Huston, 1996a). Briefly, 3 mg/kg methamphetamine was administered intraperitoneally to animals before being left in a quiet environment for 10 min. Subsequently, contra- and ipsi-lateral rotations were counted over a 10 min period and calculated as number of rotations/min. Animals which displayed solely ipsilateral turns were selected for cell implantation.

2.4. Iron oxide preparation

TAT-peptide derivatized ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles coated with dextran (IODEX-TAT-FITC; 15–20 nm) were prepared in our laboratory using the method described by Josephson et al. (1999). Briefly, the dextran-coated USPIO nanoparticles were synthesised and subsequently conjugated with TAT-fluorescein isothiocyanate (FITC) peptide [GRKKRRQRRRGYK(FITC)C-NH_2]. TAT-FITC was synthesised by the Central Research Resources unit of the Clinical Sciences Centre (Medical Research Council, London, UK), using FMOC-protected amino acid (2-(1-H-Benzotriazol-2-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HBTU) activation chemistry. The final iron concentration was 2.5 mg/mL and the solution was sterilized by γ-irradiation prior to use.